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Knocking out barriers to engineered cell activity

CRISPR-Cas9 gene-edited T cells show safety and long-term engraftment in humans

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Engineered T cell therapies are revolutionizing cancer treatment by achieving long-lasting remission in blood-related cancers, such as leukemia and lymphoma. These therapies involve removal of patient T cells, “reprogramming” them to attack cancer cells, and then transferring them back into the patient. Targeted gene inactivation (knockout) using CRISPR-Cas9 can enhance T cell activity (*1*, *2*) and has the potential to expand cell therapy applications. Until now, it has been unknown whether CRISPR-Cas9–edited T cells would be tolerated and thrive once reinfused into a human. On page XXX of this issue, Stadtmauer *et al.* (*3*) present data from a phase 1 clinical trial (designed to test safety and feasibility) on the first cancer patients treated with CRISPR-Cas9–modified T cells. The findings represent an important advance in the therapeutic application of gene editing and highlight the potential to accelerate development of cell-based therapies.

The production of engineered cell therapies involves transduction of isolated patient T cells with a disarmed virus to express a receptor recognizing an antigen present on the outside of cancer cells (through chimeric antigen receptors, CARs) or on the inside of cancer cells [through T cell receptors (TCRs) specific for cancer-associated peptides]. Once transduced, the engineered T cell population is expanded and then reinfused back into the patient. Although highly effective at treating some types of cancer, the specificity and longevity of engineered T cell activity can be improved. For example, T cell activity is naturally down-regulated through the programmed cell death protein 1 (PD-1) receptor. Systemic inhibition of PD-1 in patients can enhance T cell activity but often triggers adverse autoimmune reactions. Additionally, endogenous TCR expression competes with the transgenic receptor in engineered T cells, interfering with signaling or cell trafficking. Genetic knockout of the TCR and *PDCD1* (the gene encoding PD-1) can enhance engineered human T cell activity in preclinical human tumor xenograft models in mice (*4*, *5*). Stadtmauer *et al*. tested whether patient-derived T cells containing these gene knockouts generated by CRISPR-Cas9 are safe and persistent upon reinfusion into humans.

Six patients with either myeloma or sarcoma were enrolled in the trial, and three met the study’s criteria for T cell reinfusion. A two-step process was used to achieve both native *TCR* and *PDCD1* gene knockout and transgenic TCR expression in the engineered cells (see the figure). In the first step, isolated patient T cells were electroporated with preformed ribonucleoproteins (RNPs) of Cas9 protein and guide RNA that targets the endogenous TCR—TCR□ (*TRAC*) and TCR□ (*TRBC*)—and *PDCD1* for genetic disruption. In the second step, cells were transduced with a viral vector to express the transgenic TCR, which recognizes cancer-testis antigen 1 (NY-ESO-1), and expanded in culture to create NY-ESO-1 transduced CRISPR 3X edited (NYCE) cells. Notably, NYCE cells eliminated NY-ESO-1–expressing cells more effectively than T cells expressing the NY-ESO-1 TCR alone, as would be expected from the successful knockout of the endogenous TCR.

NYCE cells successfully engrafted in all patients and were detected up to 9 months after reinfusion. According to the authors, this persistence compared favorably to the ~1-week half-life of infused, unedited T cells expressing NY-ESO-1 TCR in previous trials. NYCE cells reisolated from a study participant also had gene expression profiles consistent with central memory cells, a mark of stable engraftment. This result contrasts with past studies in which unedited cells expressing NY-ESO-1 TCRs displayed markers of T cell exhaustion (*6*). Together, the CRISPR-Cas9 disruption of endogenous TCR and PD-1 improved the cell-killing ability of the engineered T cells and promoted long-term persistence.

But are CRISPR-Cas9–edited cells safe in humans? It has been unclear whether Cas9-edited cells will be immunogenic and whether residual Cas9—a bacterial protein—will trigger an immune response. Stadtmauer *et al*. report no editing-associated toxicity of the NYCE cells in the three patients. Furthermore, although study participants had preexisting T cells and antibodies specific for Cas9 protein [as observed previously (*7*)], antibody titers did not increase from baseline over the course of the study. The lack of a Cas9 immune response could be attributed either to immunosuppression of the patients receiving NYCE cells, or to the delivery of Cas9 as a nonviral, preformed RNP, which has a limited half-life in cells compared to viral delivery where Cas9 protein is continuously expressed in treated cells. Stadtmauer *et al*. also report minimal off-target editing by CRISPR-Cas9, and the ≤1% of NYCE cells

containing chromosomal translocations decreased in patients after reinfusion. Together, these findings provide a guide for the safe production and nonimmunogenic administration of gene-edited somatic cells.

The big question that remains unanswered by this study is whether CRISPR-edited, engineered T cells are effective against advanced cancer. Phase 1 trials assess safety, so the efficacy of the NYCE cells for treating patients was not evaluated. At the end of the study, one participant had died because of cancer progression and the other two were receiving other therapies. Although the efficacy of the Cas9-engineered cells is thus ambiguous, the authors point out that their study was restricted to editing protocols available in 2016, when the U.S. Food and Drug Administration reviewed the clinical trial application. Gene disruption efficiencies in this study were modest (15 to 45%), whereas protocols now exist for reliably achieving >90% gene disruptions in human T cells using Cas9 RNPs (*8*, *9*). Moreover, recent efforts have demonstrated CAR transgene insertion at the *TRAC* gene in human T cells, resulting in simultaneous knockout of the endogenous TCR while driving CAR expression by the native promoter (*8*, *10*). Advances in generating precise genetic modifications, as well as other choices of cancer-associated targets, could enhance the efficacy of engineered T cells for the treatment of additional cancers, including solid tumors, which have largely been resistant to the activity of engineered cell therapeutics.

The clinically validated long-term safety of CRISPR-Cas9 gene-edited cells reported by Stadtmauer *et al.* paves the way for next-generation cell-based therapies. Although the safety of other types of gene-edited somatic cells, such as stem cells, remains to be determined, encouraging results show healthy blood production in the first β-thalassemia and sickle cell anemia patients infused with cells modified by CRISPR-Cas9 (*11*). As more gene-based therapies are demonstrated to be safe and effective, the barrier to clinical translation will become cell manufacturing and administration. A restructuring of production processes for engineered cells and new CRISPR-Cas9 delivery strategies for the modification of targeted cells in the body are now imperative to reduce cost and make these revolutionary therapies accessible to all who can benefit.

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